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PREVENTION OF FORMALIN-INDUCED NOCICEPTIVE BEHAVIORS CAUSED BY PPAR-GAMMA ACTIVATION STRENGTHENED WITH CEREBELLAR TRANSCRANIAL DIRECT CURRENT STIMULATION

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Abstract

The investigation was performed on 55 male Wistar rats. The pain behaviors were induced through the formalin (2.5%) subcutaneous administration into the plantar surface of the right hind paw. Pain score value was determined continuously during 90 min.

It was established that combined usage of pioglitazone (100.0 mg/kg, i.p.) and cerebellar transcranial direct current stimulation (tDCS) (300 μA, 10.0 min) resulted in pronounced protection of pain behaviors at all stages of the pain syndrome development. The severity of investigated index decreased by 60.0% compared to the control data at 4-6 min after formalin administration ($P<0.05$). Averaged severity of pain behaviors was suppressed more than twice at Phase I and Interphase ($P<0.05$), by 60.0% at Phase 2A ($P<0.05$), and by 33.3% at Phase 2B ($P<0.05$). Pain-suppressive effect encompassed 42.0±8.25 min of Phase 2A and exceeded data in the group treated with tDCS by 39.1% ($P<0.05$) and in the group treated with pioglitazone by 64.3% ($P<0.05$).

The conclusion was made that the pain-protective effect of pioglitazone is strengthened with cerebellar tDCS.

Key words: *pain behaviors, pioglitazone, PPAR-γ, cerebellum, transcranial stimulation.*

Introduction

Pathogenesis of pain development included fundamental routes of brain functioning. Namely, bioelectrogenesis, neuromediators elaboration, and synthesis of macromolecules are contributive. A similar broad functional spectrum might be addressed to peroxisome proliferator-activated receptors (PPAR) activity [1, 2]. PPARγ recently attracted researchers' attention as those involved in pain syndrome precipitation [3-7].

It was shown that PPARγ agonists could block the production of proinflammatory cytokines, TNFα, IL-6, and IL-1β in different animal tissues as well as in patients [2, 8, 9]. Effects of PPAR agonists have been explored in animal models of inflammation, traumatic brain injury, stroke, epilepsy, and pain syndrome. Gained results revealed positive therapeutic effects and usage of PPAR agonists such as thiazolidinediones, clinically used as an antidiabetic is regarded as promising for brain disease treatment, including neuropathic pain [2, 10, 11].

Earlier, we established the role of PPARγ as significant for the antiseizure effects precipitation induced by cerebellar transcranial direct current stimulation (tDCS) [12]. Namely, blocking of PPARγ with bisphenol A diglycidyl ether (2,2'-[(1-methylethylidene) bis(4,1-phenyleneoxymethylene)] bis-oxirane, (BADGE, 100 mg/kg, i.p.) alleviate the ctDCS-caused prevention of generalized clonic-tonic fits induced with pentylenetetrazol in kindled rats. Neuroprotective effects of fastigial nucleus stimulation realized via PPARγ [13]. PPAR-γ might be activated via cAMP/PKA/PPAR γ axis [14, 15] with fastigial electrical stimulation [16]. Besides, nervus vagus anti-inflammatory action is also transduced via PPAR γ [17].

Considering that pain relief is caused by cerebellar stimulation [18-21], the ability of PPARγ agonists to relieve neuropathic pain [4, 22], present work aimed to investigate the pain syndrome manifestations in rats under conditions of cerebellar tDCS and modulation PPARγ with antagonist BADGE and agonist pioglitazone.

Methods

Experimental animals

Experiments were performed on 55 male Wistar rats with an initial body weight of 180-270 g. Animals were kept in standard conditions (constant temperature 230 C, relative humidity 60%, 12 h dark/light cycles, standard diet, and tap water were given ad libitum) and were acclimatized to laboratory conditions at least seven days before the experiment. All experiments were carried out following the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC). Before the study, the experiments were approved by Odesa National Medical University Bioethics Committee (UBC) (approval No. 5 dated 5/04/2019).

Formalin test

Rats were put in a plastic transparent observation chamber (height 50 cm Width 45 and Length 50 cm) for their habituation for 60 min. After that, 50μL of 2.5% formalin was injected subcutaneously into the plantar surface of the right hind paw with a 30 gauge needle. Video recording (smartphones) of pain behaviors started immediately after injection and continued for 90 min.

Pain behaviors were scored as follows: 0, the injected paw was not favored; 1, the injected paw had little or no weight placed on; 2, the injected paw was elevated and not in contact with any surface; and 3, the injected paw was licked or bite. Scores were continuously observed for the experiment duration (90 minutes). Off-line analysis of data was performed by the modified method [23]. Namely, a score of pain behaviors calculated for 3-minutes intervals and three phases of pain behaviors were identified [24, 25].

Cerebellar tDCS

Cerebellar stimulations were performed in accordance with the earlier described method [12]. In short, the cathode (diameter 3.5 mm) was fixed with an adhesive tape on the skull midline caudally with respect to the lambda; this provided the orientation of stimulation to the cerebellum. A conductive gel was preliminarily applied below the electrode on the depilated skin. The anode (40×45 mm) was placed on the rat's abdominal surface. The current intensity was 300 μA. Stimulation was performed for 10 min and the modified generator, ETRANS (FSU), served as the source [12]. Formalin test was performed 10 min after termination of tDCS.

Groups of animals

Nine rats treated with DMSO i.p. and pseudo–stimulated were used as a control. Experimental groups of rats included rats with tDCS, treated with BADGE (100.0 mg/kg, i.p.), treated with tDCS and BADGE (100.0 mg/kg, i.p.), treated with pioglitazone (100.0 mg/kg, i.p.) and combined usage of tDCS and pioglitazone (100.0 mg/kg, i.p.) (total – 46 rats).

Investigated compounds administration

Pioglitazone (Lilly S.A., Spain) was administered in 0.3-0.5 DMSO solution in a dose of 100.0 mg/kg, i.p. in 60.0 min before formalin test. The antagonist of PPARγ, BADGE (Santa Cruz Biotechnology, USA), was i.p. injected in the dose of 100 mg/kg 45 min before tDCS.

Statistics

Scores of pain behaviors were analyzed using repeated-measures ANOVA. The comparisons between experimental and control groups at each 3 minutes interval were estimated using Tukey's posthoc test. Also, the averaged data for each phase of pain behaviors were compared using one-way ANOVA and Newman-Keuls test. The Shapiro-Wilk test for normality was used. *P* values <0.05 were considered significant. Data are presented as Mean ±SD.

Results

Subcutaneous formalin administration (2.5%) induced nociceptive behaviors in acute (phase 1- from the first to the 7th min) and chronic, lasting during all periods of observation (90 min). The nociceptive behaviors were alleviated during interphase (8-14 min). During this period, such manifestations as biting or licking the injected paw substantially attenuated or disappeared. The second phase began approximately 15 min after the formalin injection and lasted until 90 min (Fig.1).

After tDCS, the tendency to decreasing of the pain behaviors was noted at the very beginning of observation with the significant diminution during a short period (6-9 min) by 31.5% (P<0.05) (Fig.1, A). Also, long (25-51 min) significant reduction of nociceptive behaviors – by 26.7%-37.7% registered during Phase 2A.

Averaged values of the severity of pain behaviors were significantly reduced during Phase 2A – by 17.2% when compared with the control data (P<0.05) (Fig.1, B).

The nociceptive behaviors after BADGE (100.0 mg/kg, i.p.) administration were characterized by the reduction of its severity during 7-12 min after formalin administration by 31.4-35.0% in comparison with the control data (P<0.05) (Fig.2, A). Also, the reduction by 26.7% (P<0.05) was registered at a 24-27th period while at 75-81^{t} min the significant increase of pain behaviors was seen – by 33.3-42.3% when compared to control data (P<0.05) (Fig. 2, A). The averaged values of the severity of the nociceptive behaviors did not differ from control values (P>0.05) (Fig.2, B).

Two short-time significant reductions of pain behaviors severity were seen under conditions of combined usage of tDCS and BADGE (100.0 mg/kg, i.p.). It was diminished by 25.7% at 6-9 min and by 25.3% at 21-24 min from the moment of formalin administration (P<0.05) (Fig.3, A). The averaged values of the severity of pain behaviors were free from significant differences compared to the control data (Fig.3, B).

Pioglitazone administration caused a substantial reduction (by 26.8-50.0%) of the pain behaviors severity at the period starting from the first 3 min till 12th min from the moment of formalin administration (Fig.4, A). During 15-18th min, the reduction was also significant (44.0%) and starting from 21t min stable period of severity reduction (28.0- 41.3%) lasted till 36th min of observation. Significant reduction of pain behaviors severity was registered during 63^{d} - 75th min (by 36.4-57.9%) (P<0.05) (Fig.4, A). Averaged values of the investigated index revealed a significant decrease in pain behaviors severity at Phase I by 20.0% (P<0.05) (Fig.4, B). Also, the reduction was 22.9% at Phase 2A (P<0.05) and 44.4% at Phase 2B (P<0.05).

Combined usage of tDCS and pioglitazone (100.0 mg/kg, i.p.) resulted in pronounced protection of pain behaviors at all stages of the formalin-induced pain syndrome development (Fig.5, A). The severity of investigated index decreased by 60.0% when compared to the control data at 4-6 min after formalin administration (P<0.05) (Fig.5, A). Pain-suppressive effect encompassed almost the whole Phase2A (up to 55-57th min). The maximal reduction was at 34-36th min – by 75.0% and 25-27th min – by 71.1% (P<0.05). Averaged severity of pain behaviors was suppressed more than twice at Phase I and Interphase (P<0.05), by 60.0% at Phase 2A (P<0.05), and by 33.3% at Phase 2B (P<0.05) (Fig.5, B).

The most durable protection of pain behavior caused with both tDCS and pioglitazone was observed during Phase2A. The duration of such effect in rats with a combined usage of tDCS and pioglitazone was 42.0±8.25 min and exceeded data in the group treated with tDCS by 39.1% (P<0.05) and in the group treated with pioglitazone by 64.3% (P<0.05) (Fig.6).

Discussion

Hence, gained data showed that tDCS of the cerebellar surface caused suppression of formalin pain behaviors starting from Phase1, which is most pronounced at Phase 2A. Pain protective effects of cerebellar tDCS are alleviated with BADGE administration, which supports PPAR-gamma involvement. Besides, pioglitazone caused well-verified pain–protective action at first and second phases, and these effects increased under conditions of combined usage of cerebellar tDCS and pioglitazone.

Cerebellar stimulation causes two types of responses: precipitates immediately with the stimulation (short-term precipitation) and delayed ones [26]. In our experimental conditions, it is expected that the outer cerebellar surface, more precisely – Purkinje cells located at lobules V-VII are most affected with electrodes applied to the posterior skull zone. Optogenetic data revealed that the increased activity of Purkinje cells induces "phasic" or "online" effects [27-30]. Depolarization of Golgi inhibitory neurons is responsible for long-lasting changes [26]. Hence, cathodal depolarization of Golgi cells and inhibition of Purkinje cells need a time for the consequent renovation and "overshoot" of initial cellular activity with postponed precipitation of pain suppression. Purkinje cells represent the sole output from the cerebellar cortex and inhibit the dentate nucleus, ultimately dampening cortex excitability, including pain-sensitive neurons. Such neurophysiological assumptions explain the more pronounced effects revealed at Phase2 of pain formalin syndrome. Besides, proinflammatory cytokines' role in Phase2 development [1-3, 11, 23-25] is also targeted with pioglitazone and tDCS.

It is important to note that centrally acting drugs effectively suppress both phases of pain behaviors in the formalin test [31, 32]. Those compounds, which cause peripheral effects such as nonsteroidal anti-inflammatory drugs - acetylsalicylic acid, ameliorate only the second phase [31, 32]. Our results clearly show that tDCS and pioglitazone effectively prevented pain behaviors in both phases. Mansouri M.T. et al. [11] delivered a similar result on pioglitazone effects. Hence, the role of centrally located PPARγ is assumed as a target point for their pain-protective synergy effects.

Meanwhile, the involvement of central PPARγ as an entire route for antinociceptive action of cerebellar tDCS is hardly suspect. Thus, considering the role of neurotransmission via dorsal horn gate as a main for the pain precipitation at the first phase [33], it is worth assuming that descending influences from irritated cerebellar structures contribute a lot to pain suppression. Besides, central effects are of significance as far as the cerebellum modulates the activity of limbic structures [28, 30, 34, 35]. Both central and peripheral effects of cerebellum realized via endogenous opioid system involvement [35, 36, 37], inhibition of proinflammatory cytokines (TNF-α, IL-1β) elaboration [38], reduction of oxidative stress [39]. GABA and glutamate level, which is considered as a target for peripheral analgetics action [11], is also modulated [40].

Besides, the synergy between rapamycin and pioglitazone [41, 42] and antinociceptive effects of rapamycin [43] point to the possible involvement of mTOR-dependent mechanisms in the realization of observed effects combined usage tDCS and pioglitazone against formalin pain behaviors.

The second phase of the formalin test has resulted from a peripheral inflammatory process. Hence, the established effect of tDCS and pioglitazone synergy should translate to other forms of brain pathology with pro-inflammatory pathogenesis.

Conclusions

1. Pioglitazone (100.0 mg/kg, i.p.) alleviates both the first and second (2A) phases of formalin pain behaviors.

2. Cathodal cerebellar tDCS is effective against the second phase of formalin-induced pain in rats, and the effects of tDCS are blocked with BADGE administration.

3.Pain-protetcive pioglitazone action stregthened by cerebellar tDCS.

Acknowledgments

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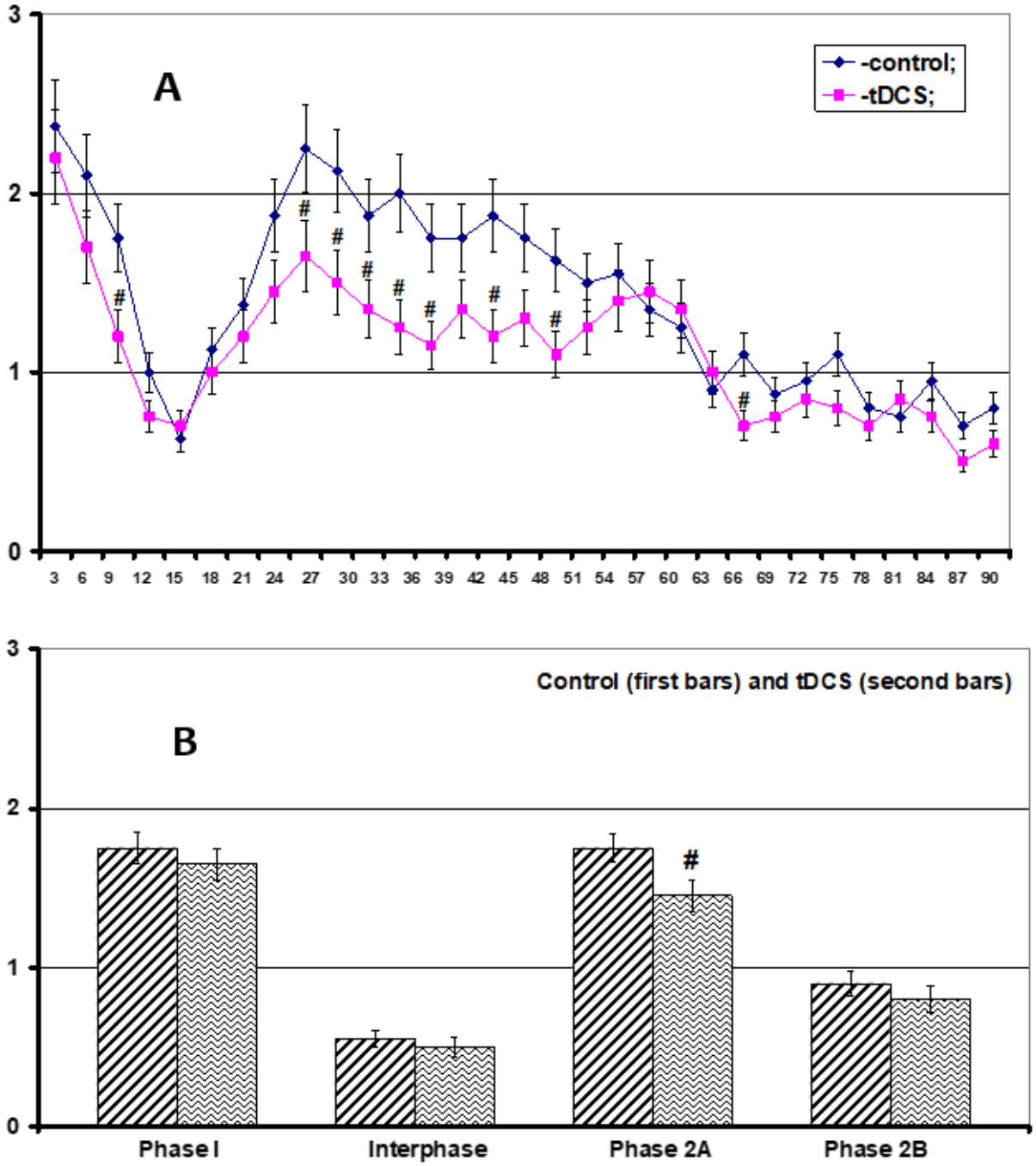
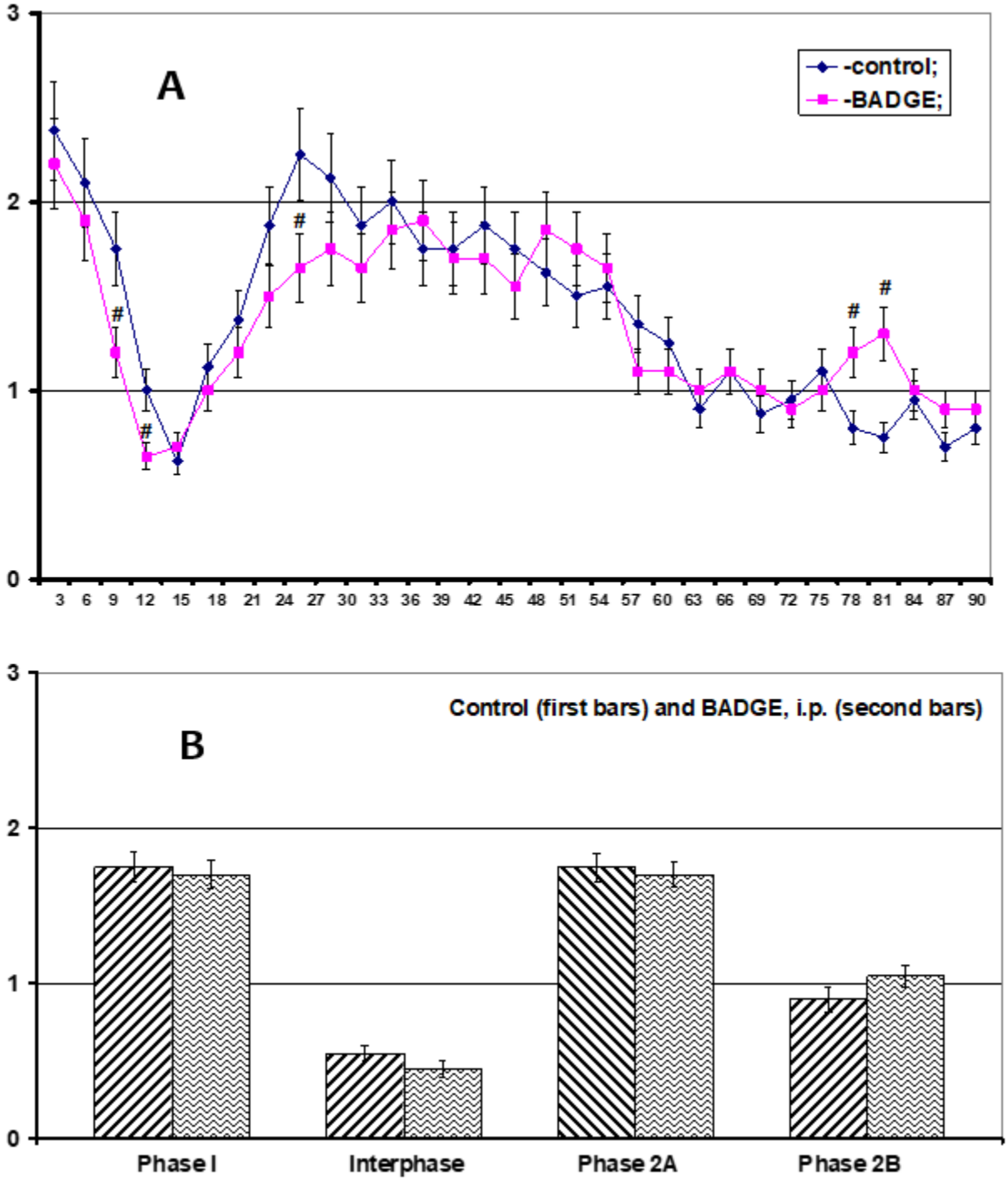
Figure 1



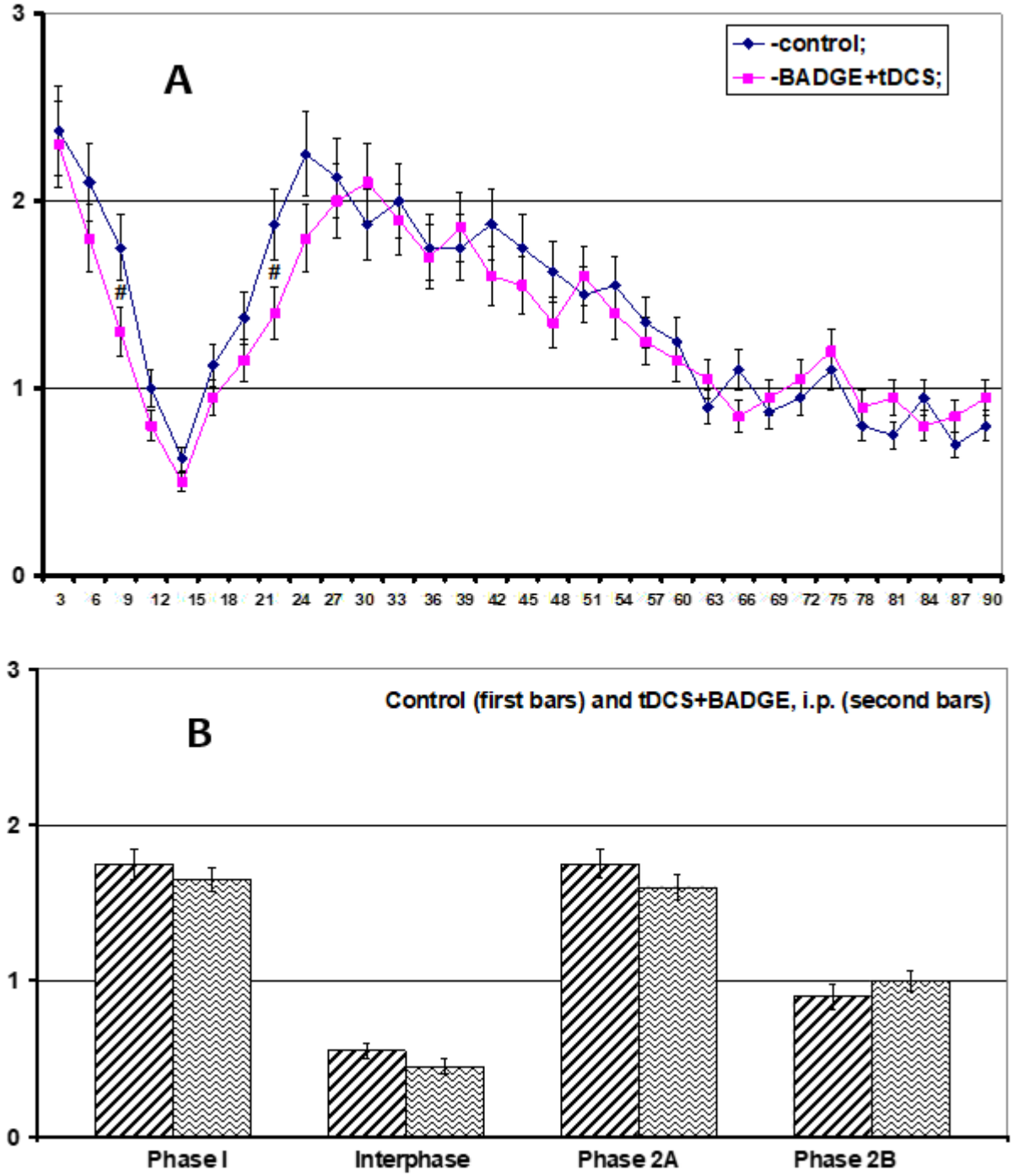
Time – course of pain behavior induced with 2.5% formalin intraplantar administration to control sham-stimulated rats and rats with cerebellar tDCS.
A: abscissa – time (minutes) from the moment of formalin injection. Ordinate – the severity of pain behaviors (scored in balls);
B: averaged severity of pain behaviors corresponding with pain syndrome development phases: Phase I – 1-7 min, Interphase – 8-14 min, Phase 2A- 15-60 min, and Phase 2B- 61-90 min after formalin administration.
Data are presented as M±SD; #-P<0.05 compared to the control data.

Figure 2



Effect of BADGE (100.0 mg/kg, i.p.) administration on time–course of pain behavior induced with 2.5% formalin intraplantar administration.
N o t e s : the same as in Fig.1.
Data are presented as M±SD; #-P<0.05 in comparison with the control data.

Figure 3

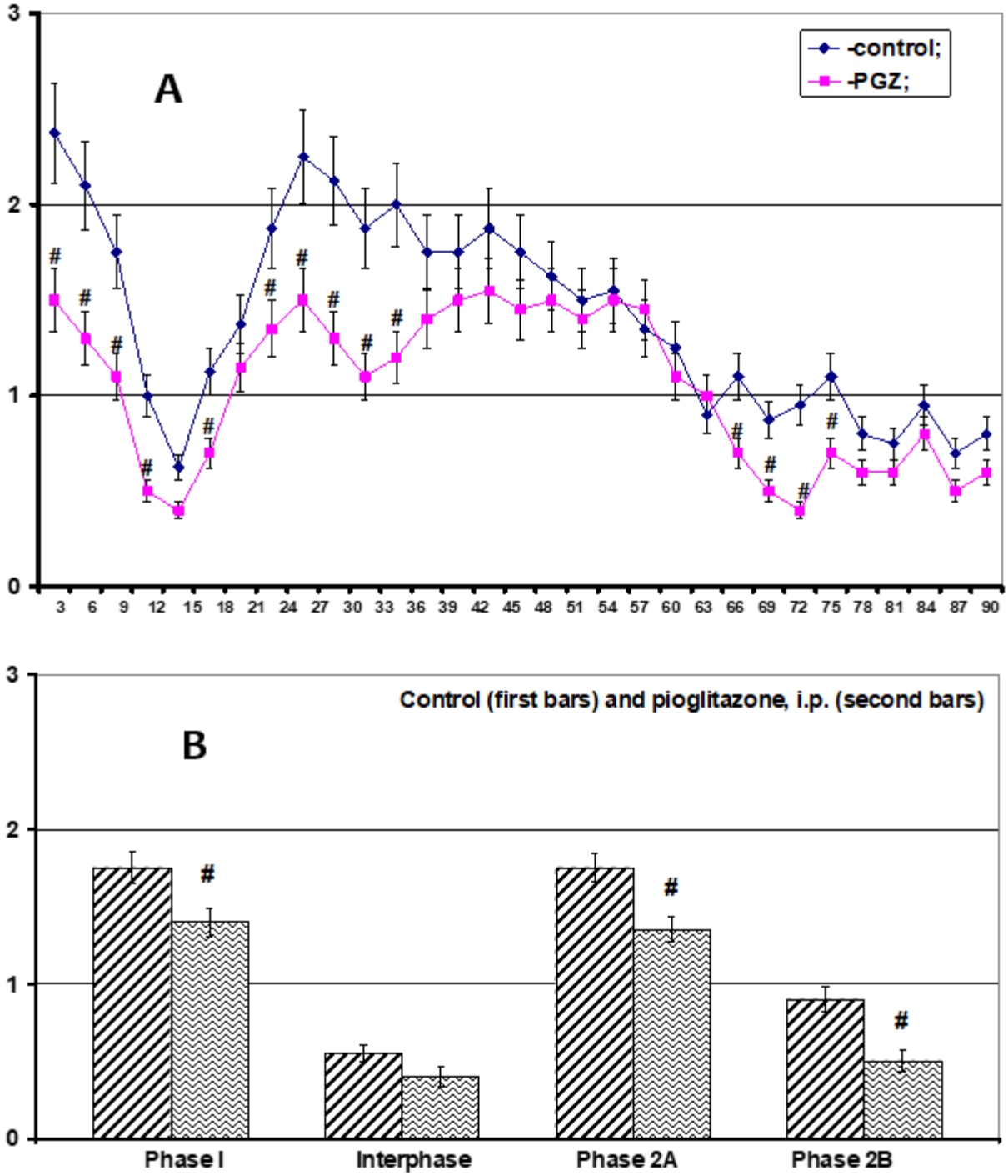


Effect of tDCS performed after BADGE (100.0 mg/kg, i.p.) administration on the time – course of pain behavior induced with 2% formalin intraplantar administration.

N o t e s : the same as on Fig.1.

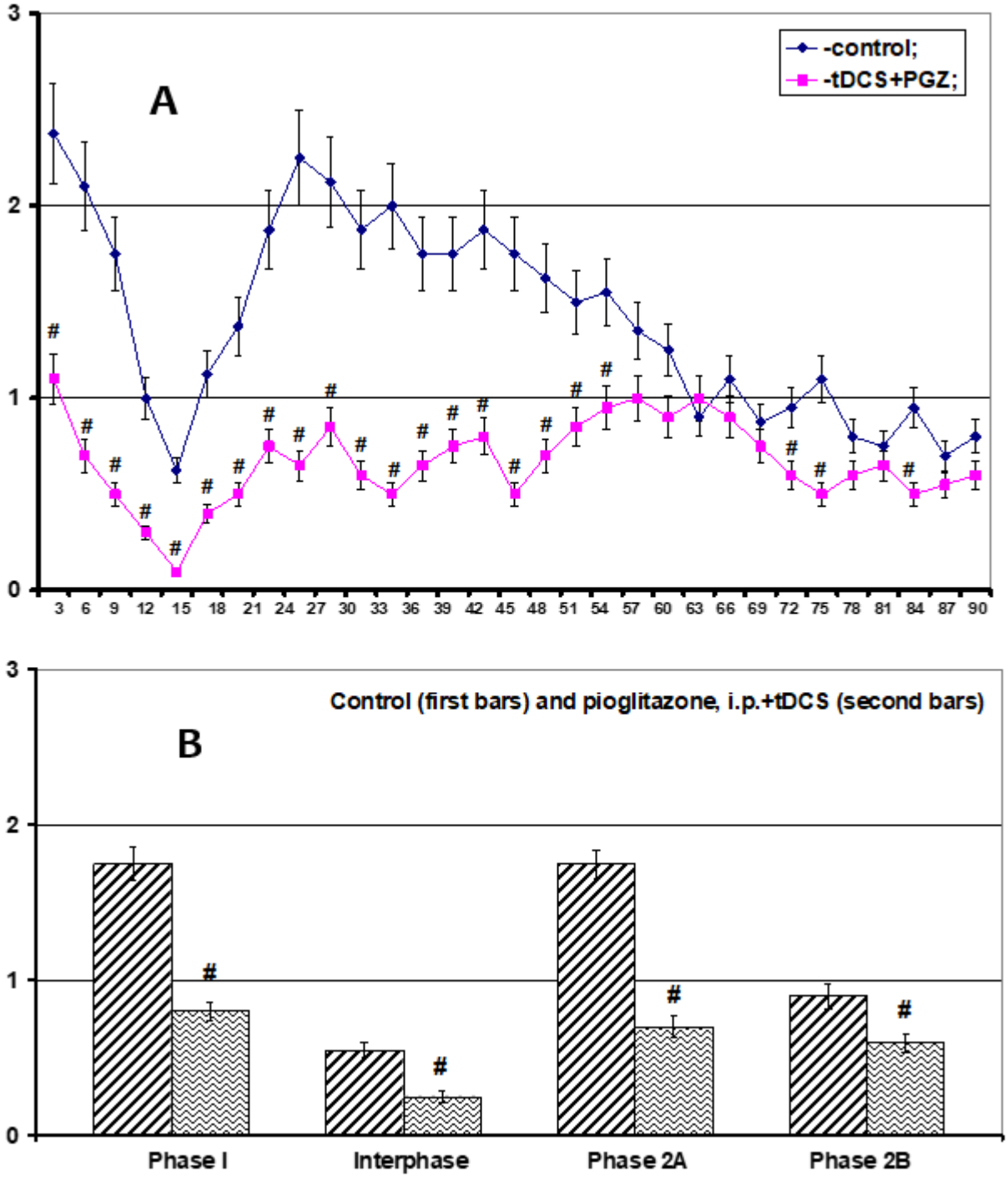
Data are presented as M±SD; #-P<0.05 in comparison with the control data.

Figure 4



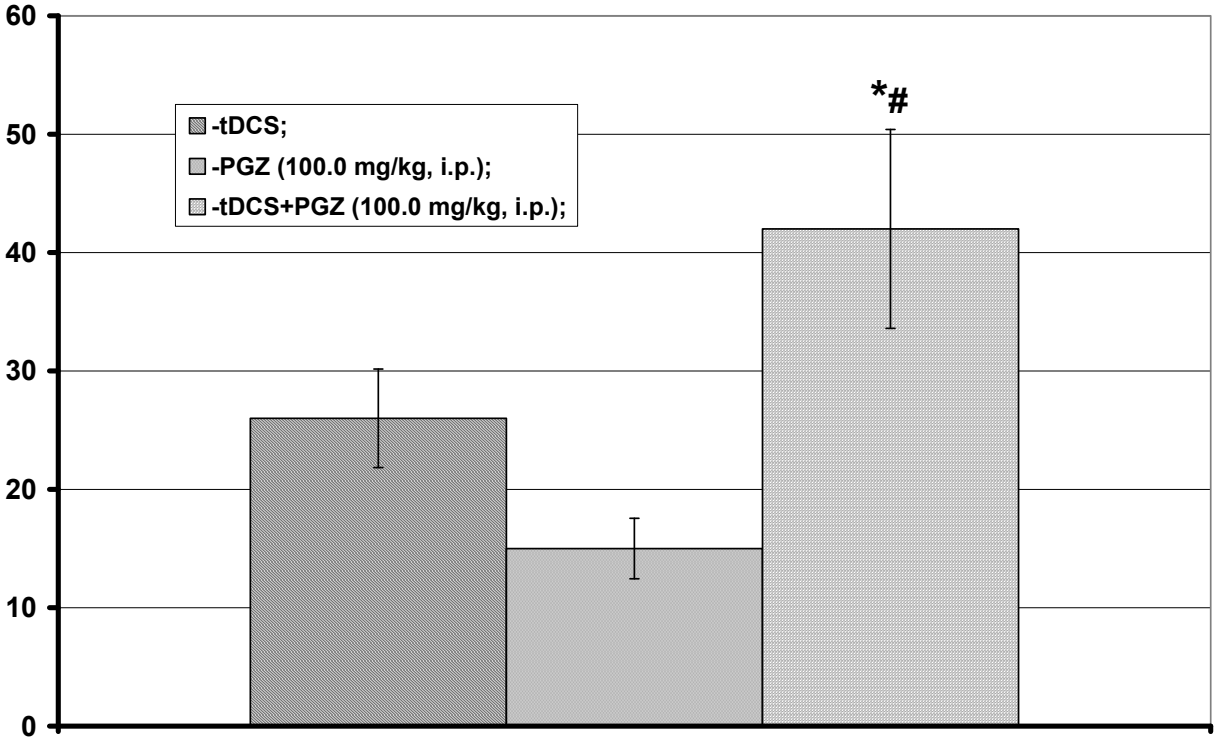
Effect of pioglitazone (PGZ) (100.0 mg/kg, i.p.) administration on time–course of pain behavior induced with 2.5% formalin intraplantar administration.
N o t e s : the same as in Fig.1.
Data are presented as M±SD; #-P<0.05 in comparison with the control data.

Figure 5



Effect of combined usage of tDCS and pioglitazone (PGZ) (100.0 mg/kg, i.p.) on time–course of pain behavior induced with 2.5% formalin intraplantar administration.
N o t e s : the same as in Fig.1.
Data are presented as M±SD; #-P<0.05 in comparison with the control data.

Figure 6



Influence of pioglitazone (PGZ) and tDCS on the life span of pain behaviors (Phase 2A).
N o t e s: abscissa – observation groups; ordinate – minutes. Data are presented as M±SD; *-P<0.05 vs. group with tDCS; #-P<0.05 vs. group treated with PGZ.